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<p>(21) International Application Number: PCT/US93/03603 (22) International Filing Date: 16 April 1993 (16.04.93) (30) Priority data: 07/871,139 20 April 1992 (20.04.92) US (71) Applicant: MALLINCKRODT SENSOR SYSTEMS, INC. [US/US]; 1230 Eisenhower Place, Ann Arbor, MI 48108 (US). (72) Inventor: HENSON, Ralph ; 1878 Great Highway, San Francisco, CA 94122 (US). (74) Agents: CITKOWSKI, Ronald, W. et al.; 3001 W. Big Beaver, Suite 624, Troy, MI 48084 (US).</p>		<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i></p>
<p>(54) Title: CALIBRATION REAGENT HAVING A STABILIZED CONCENTRATION OF DISSOLVED GAS</p> <p>(57) Abstract</p> <p>A calibration reagent of the type including known concentrations of one or more gases dissolved in a carrier vehicle further includes helium dissolved therein. The helium acts to stabilize the concentration of dissolved gases. The reagents are very useful in the calibration of a wide variety of gas analysis equipment and may be readily stored under a wide range of ambient conditions.</p>		

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- 1 -

**CALIBRATION REAGENT HAVING A STABILIZED
CONCENTRATION OF DISSOLVED GAS**Field of the Invention

This invention relates generally to the calibration of analysis equipment. More specifically, the present invention relates to calibration reagents including dissolved gases therein. Most specifically, the present invention relates to reagents for calibrating blood gas analysis systems.

10 Background of the Invention

Automated chemical analyses are rapidly supplanting manual techniques, particularly in the health care field. The rapid and accurate analysis of blood chemistry is particularly important in operating rooms, critical care facilities and clinical environments.

It is necessary to calibrate analysis systems in order to assure the accuracy and reliability of their results. Calibration is typically carried out by the use of reagents which are facsimiles of the materials being analyzed and which include known quantities of the target analytes therein. For example, equipment for blood gas analysis is typically calibrated by utilizing a blood facsimile which includes known concentrations of dissolved oxygen and carbon dioxide therein and which may further include bicarbonate, calcium, sodium and potassium ions, as well as other ionic species and organic species such as glucose.

- 2 -

There are many problems associated with the use of liquid based calibration reagents which contain dissolved gases. The concentration of a given gas in a liquid will vary depending upon the ambient temperature and pressure conditions to which the liquid is exposed. Furthermore, atmospheric gases can dissolve into the liquid thereby changing the concentration of particular species therein.

One approach to the problem of providing accurate calibration reagents for blood gas analysis equipment involves the on site preparation of the reagents by the tonometry of liquid with a known concentration of gas. In this process, a gas is bubbled through a liquid under controlled conditions; the concentration of gases in the reagent may be calculated after compensating for temperature and barometric pressure, and the solution is ready for use in a calibration procedure. Solutions thus prepared must be used very promptly since their gas concentration can change rapidly.

Another approach to the problem of calibration solutions involves the use of previously prepared solutions which are stored in a flexible, relatively gas-impermeable package having zero headspace. By zero headspace is meant that the liquid occupies the entirety of the package and there is no free gas therein. In preparing reagents of this type, care must be taken to avoid the introduction, or formation during storage, of any bubbles since they can change the concentration of gases in the reagent and can also interfere with the analysis itself. Typically,

- 3 -

solutions of this type are packaged at total gas pressures substantially less than atmospheric and at elevated temperatures. One package of this type is disclosed in U.S. Patent No. 4,116,336 of Sorensen et al. The reagent
5 described therein is a blood facsimile which is packaged in a laminate foil-polymer bag, at a total gas pressure of less than 600 and preferably 500-550, mmHg at 37°C.

A major problem encountered with the flexible bag approach is that the package is responsive to ambient
10 temperature and pressure changes and hence storage at high altitudes, air transport or thermal cycling can cause outgassing and bubble formation. In order to minimize such problems, the total gas pressure is kept substantially below atmospheric, typically in the range of 500-550 mmHg.
15 This very low pressure gives rise to various problems. Most importantly, the low pressure within the bag facilitates the diffusion of ambient atmospheric gases therethrough, hence causing a change in the composition of the reagent during storage. Although great care is taken
20 in selecting gas-impermeable packaging materials, it has been found very difficult to provide a total barrier to diffusion. Also, filling of the bags at low dissolved gas pressures necessitates additional care and increases the cost of production. Another approach involves filling the
25 bags at higher pressures, typically 650 mm or more. While filling is simplified, problems of outgassing during storage are exacerbated. This approach is disclosed, for example, in U.S. Patent 4,871,439.

- 4 -

It is desirable to have a storage-stable package of calibration reagent for blood gas analyzers and other dissolved gas analysis systems. The calibration reagent should resist outgassing even when stored under extreme ambient temperature and pressure conditions and it should be simple and economical to prepare.

Brief Description of the Invention

As will be disclosed in greater detail hereinbelow, the present invention provides an improved calibration reagent and method for its preparation. The reagent comprises a liquid, such as a blood facsimile, having one or more gaseous materials dissolved therein. The calibration reagent is prepared at total dissolved gas pressures which are near, or only slightly below, atmospheric; however, it is resistant to bubble formation during storage. The reagent retains its stated composition during storage at high altitudes and at temperature extremes.

There is more specifically disclosed herein a calibration reagent for a gas analyzer. The reagent comprises a liquid vehicle having a known concentration of a first gaseous material dissolved in the vehicle and further including helium dissolved therein. Helium has the unique property of being more soluble in warm liquid than in cold liquid. The first gaseous material may comprise oxygen or carbon dioxide and in particular embodiments, the reagent may include a plurality of gaseous materials dissolved therein.

- 5 -

The reagent may comprise a calibration reagent for a blood gas analyzer and in such instance the reagent may include oxygen in a range of concentrations sufficient to create a partial pressure of 50-300 mmHg. The reagent
5 may also include a range of concentrations of carbon dioxide sufficient to produce a partial pressure of 10-100 mmHg therein. In yet further embodiments, the reagent may include dissolved ionic materials such as bicarbonate ion, sodium ion, potassium ion and the like.

10 The reagent is preferably supplied in a flexible package having zero head space. The package is fabricated from a material having a low permeability to gases and particularly from a material having a higher permeability for helium than for oxygen, carbon dioxide and nitrogen.

15 Brief Description of the Drawings

FIGURE 1 is a graph depicting the pressure of dissolved gas in reagent package of the present invention as a function of storage time; and

FIGURE 2 is a cross sectional view of a flexible
20 package of calibration reagent structured in accord with the principles of the present invention.

Detailed Description of the Invention

The calibration reagents of the present invention include a liquid vehicle together with a known
25 concentration of one or more gaseous materials therein and further include helium dissolved in the vehicle. As a result of this formulation, the calibration reagents retain

- 6 -

their compositional integrity over relatively long periods of time and are not detrimentally affected by changes in pressure or temperature during storage. Helium is an extremely inert substance which is generally not included
5 in reagents for chemical analyses; however, helium manifests unique properties and the present invention recognizes that these properties make helium an advantageous additive for stabilizing the compositional integrity of solutions having gases dissolved therein.

10 Because of its inertness to all commonly encountered reagents, helium will not adversely interfere with calibration or analytical procedures. Helium also possesses unique solubility properties. In contradistinction to most gases, helium is more soluble in
15 hot water than in cold water. At atmospheric pressure approximately 9.4 cc of helium dissolves in 1 liter of cold (0°C.) water while approximately 10.5 cc of helium dissolves in 1 liter of hot (50°C.) water. These figures also make clear the fact that helium has a very low solubility in
20 water and hence relatively small amounts of dissolved helium can create a relatively large partial pressure. Finally, helium has an extremely high mobility and is far more volatile than any other gases present in calibration reagents. All of these unique properties of helium
25 synergistically interact to stabilize dissolved-gas-containing calibration reagents toward compositional change. Bubble formation occurs in a liquid when the total pressure of the gases dissolved therein exceeds the atmospheric pressure. Bubble formation can occur in

- 7 -

calibration reagents when they are heated since most gases become less soluble in hot liquids. Since helium is more soluble in warm liquids than in cold liquids, inclusion of helium in a gas-containing calibration reagent tends to
5 inhibit bubble formation at elevated temperatures. As the reagent is warmed, there is tendency for most dissolved gases to increase their partial pressures and if the total gas pressure becomes too high, bubble formation will occur; however, if helium is included in the reagent, it will
10 decrease its partial pressure as the temperature increases thereby preventing bubble formation.

The fact that helium has a very low solubility in aqueous based liquids further operates to its advantage since relatively small amounts of dissolved helium will
15 exert a fairly large partial pressure; hence, the effects of helium are magnified by its low solubility. If the ambient pressure becomes sufficiently low so as to be less than the total dissolved gas pressure in the liquid, bubble formation will still occur; however, the generation of an
20 extremely small helium bubble will significantly lower the total pressure of dissolved gas thereby precluding further bubble formation. Thus it will be seen that helium tends to inhibit bubble formation by decreasing the total pressure of dissolved gases in a calibration liquid as the
25 temperature increases and further acts to mitigate the effects of any possible bubble formation that may occur.

The very high mobility of helium still further enhances the stability of gas-containing calibration reagents. The permeability of a large number of materials

- 8 -

to helium is far higher than it is for most other gases. In a typical calibration reagent, such as one which might be employed for a blood gas analyzer, there is present a partial pressure of oxygen which is generally no more than 5 300 mm Hg and a partial pressure of carbon dioxide which usually does not exceed 100 mm Hg. In prior art reagents the remainder of the atmosphere was made up of nitrogen and the total gas pressure was usually kept below 550 mm Hg to avoid bubble formation. Even though great care is taken to 10 employ packaging materials which have very low gas permeabilities, some leakage will occur during storage and since the total pressure in the package is relatively low, atmospheric gases will tend to leak into the package. By the use of helium, as was set forth hereinabove, total gas pressures within the reagent package can be near, or only 15 slightly less than, atmospheric and leakage into the package is minimized. Furthermore, the greater permeability of the package to helium in contrast to oxygen and nitrogen further stabilizes the composition.

20 Figure 1 illustrates this phenomenon. Shown in the figure is a graph depicting pressure of the dissolved gases as a function of time. Curve A depicts the total gas pressure in a calibration reagent which includes helium and a known concentration of dissolved oxygen. The reagent is 25 contained in a package which has a higher permeability for helium than for oxygen or nitrogen. It will be noted that the total gas pressure manifests an initial drop followed by a slow rise. This corresponds to helium leaking out of the package and being replaced by nitrogen which leaks in.

- 9 -

The two processes occur simultaneously, but the rate of helium leakage is faster. The low point on Curve A represents the depletion of helium and the slow rise in total gas pressure is attributable to continued inward
5 diffusion of nitrogen. The package is initially filled at a total gas pressure which is near atmospheric and because of the solubility properties of helium, bubble formation is initially inhibited. As the total gas pressure decreases, the tendency to bubble formation is further inhibited. As
10 the helium is being replaced by nitrogen, the tendency to bubble formation does increase; however, bubble formation becomes a real concern only when the total gas pressure of the nitrogen containing solution becomes a significant fraction of atmospheric pressure.

15 Curve B represents the concentration of dissolved oxygen and throughout the entire process it will be noted that the concentration is essentially stable. Since the concentration of oxygen in the solution is approximately that of a solution equilibrated with ambient air, and since
20 the package has a low permeability to oxygen, leakage of oxygen into or out of the package is fairly minimal. The time scale for diffusion of the helium and nitrogen will depend upon the permeability of the package, the nature of the reagent and the actual pressure of the gases. It has
25 been found that a typical calibration solution of the type which will be described in greater detail hereinbelow, reaches a helium depletion point after approximately four months and manifests a sufficiently long pressure rise time that a total useful life of approximately two years is

- 10 -

achieved before bubble formation becomes a significant concern.

As mentioned hereinabove, the calibration reagent of the present invention is most advantageously packaged in a flexible package having zero head space. By zero head space is meant that a liquid entirely fills the package without any bubbles or voids. Referring now to Figure 2 there is shown, in cross section, one particular reagent package 20 structured in accord with the principles of the present invention. The package is fabricated from a relatively low gas permeability material comprising a laminate of metallic foil 22 interposed between layers of thermo plastic polymer 24,26. The packet is fabricated by laminating the interior layers 26 of polymeric material by a heat sealing process so as to provide a packet which contains the calibration reagent 28 of the present invention therein. It is to be understood that the reagent of the present invention may be packaged in other manners than as is shown herein. For example, the packaging may be totally, or partially rigid, with zero head space.

In addition to dissolved gases, it is generally preferable that the calibration reagents include other species such as known concentrations of potassium, calcium, bicarbonate, sodium and other such ions. Additionally, the reagent will preferably include a buffer to establish a stable pH, and may include other species such as glucose or colorimetric standards. The specific quantities of each of the foregoing species present in the solution will depend upon a particular applications and the particular equipment

- 11 -

being calibrated. The following examples detail some specific compositions of calibration reagent for a blood-gas analyzer and the methods by which they are manufactured.

5 Example 1

This calibration reagent was prepared to approximate a blood sample and is intended for use in calibrating a carbon dioxide sensor. The solution is water based and Table 1 hereinbelow sets forth the concentrations
10 of the dry and liquid components of the solution.

TABLE 1

	<u>COMPOUND</u>	<u>CONCENTRATION</u>	<u>GRAMS/LITER</u>
	MOPS ACID	60 mmol/l	12.588g
	NaMOPS	25 mmol/l	5.672g
15	NaHCO ₃	10 mmol/l	0.840g
	Na ₂ SO ₃	42 mmol/l	5.294g
	NaCl	57 mmol/l	3.331g
	KCl	2.0 mmol/l	0.149g
	CaCl ₂ 2H ₂ O	0.25 mmol/l	0.037g

20 The calibration solution was prepared by dissolving the ingredients of Table 1 in distilled water. The solution thus prepared was heated to 37°C. and tonometered at 700 mm Hg absolute pressure with a gas mixture comprising 10%
25 carbon dioxide in helium. Tonometring was carried out until saturation was achieved. The solution was then packaged in a flexible bag similar to that set forth in Figure 2.

The solution was analyzed and found to have the
30 following properties: pH 6.890 - 6.910; pressure of CO₂,

- 12 -

63-67 mmHg; pressure O_2 , 0.0mmHg; potassium concentration as measured by a Radiometer brand analyzer 1.8 -1.9 mmol/l; potassium ion concentration as measured by a Nova brand analyzer 1.83 -1.98 mmol/l and a calcium ion concentration of .18 - .22 mmol/l.

Example 2

A second calibration solution was prepared which included both oxygen and carbon dioxide therein. The liquid and solid components of the calibration solution are set forth in Table 2.

TABLE 2

COMPOUND	CONCENTRATION	GRAMS/LITER
Buffer, $NaHCO_3$	20.0 mmol/l	1.680 g
15 NaCl	110 mmol/l	6.430 g
KCl	6.0 mmol/l	.447 g

In order to prepare the calibration solution, the ingredients set forth in the table were dissolved in distilled water, pH was adjusted to approximately 7.3 by the use of 1.0 N HCl and the resultant solution was tonometered with a gas mixture comprising 21% O_2 and 6.3% CO_2 in helium. Tonometry was carried out at 37°C. and 700 mm Hg absolute pressure. As in the preceding example, the resultant tonometered solution was sealed in an air tight, zero head space package. Analysis of the calibration solution of Example 2 indicated the following properties: pH 7.330 - 7.345; pressure of CO_2 37.0 - 41.0 mm Hg;

- 13 -

pressure O₂ 135 -140 mm Hg; potassium ion concentration 5.6 - 5.8 mmol/l as measured by a Radiometer brand apparatus; and a concentration of potassium ion of 5.60 - 5.75 mmol/l as measured by a Nova brand apparatus.

5 It has been found that the solutions as prepared herein above exhibit a long shelf life. The concentration of the various species, particularly the gas concentrations are not changed if the packages are stored at high altitudes or exposed to temperature extremes.

10 Various other compositions may be prepared in accord with the principles of the present invention. For ease of processing and handling, the total gas pressure within the packages is most advantageously near, or slightly below atmospheric.

15 Although the foregoing has primarily been concerned with the preparation of calibration reagents which are blood facsimiles used in calibration of blood gas analysis equipment, it is to be understood that the present invention is not so limited and that it may be employed to
20 prepare a variety of calibration reagents including dissolved gases therein. Toward that end it will be appreciated that the foregoing drawings, discussion and description are merely illustrative of particular embodiments of the present invention and not limitations
25 upon the practice thereof. It is the following claims, including all equivalents, which define the scope of the invention.

- 14 -

Claims

1. A packaged calibration reagent for a gas analyzer comprising:
 - a liquid vehicle;
 - 5 a known concentration of a first gaseous material dissolved in said vehicle;
 - helium dissolved in said vehicle; and
 - a flexible container having a higher permeability for helium than for: said first gaseous material, oxygen,
10 carbon dioxide and nitrogen;
 - said liquid vehicle, together with the dissolved helium and the dissolved first gaseous material being sealed in said container with zero head space.
2. A reagent as in claim 1, wherein said first
15 gaseous material is oxygen.
3. A reagent as in claim 2, wherein oxygen is present at a partial pressure of between 50-300 mm Hg.
4. A calibration reagent as in claim 1, further including a known concentration of a second gaseous
20 material dissolved in said vehicle.
5. A calibration reagent as in claim 4, wherein said second gaseous material is carbon dioxide.

- 15 -

6. A calibration reagent as in claim 5, wherein the carbon dioxide is present in an amount sufficient to create a partial pressure of 10-100 mm Hg.

7. A calibration reagent as in claim 1, wherein
5 the total pressure of gases dissolved therein is below 760 mmHg at 37°C.

8. A calibration reagent as in claim 1, wherein said liquid vehicle is water.

9. A calibration reagent as in claim 1, wherein
10 said liquid vehicle includes bicarbonate ion dissolved therein.

10. A calibration reagent as in claim 1, wherein said liquid vehicle includes a buffer for maintaining the pH of the reagent in the range of 6.8-7.5.

15 11. A calibration reagent as in claim 1, further including calcium and potassium ion dissolved in said vehicle.

12. A packaged reagent for calibrating a blood gas analyzer comprising:

20 a flexible container which has a greater permeability for oxygen, carbon dioxide and nitrogen than it does for helium;

- 16 -

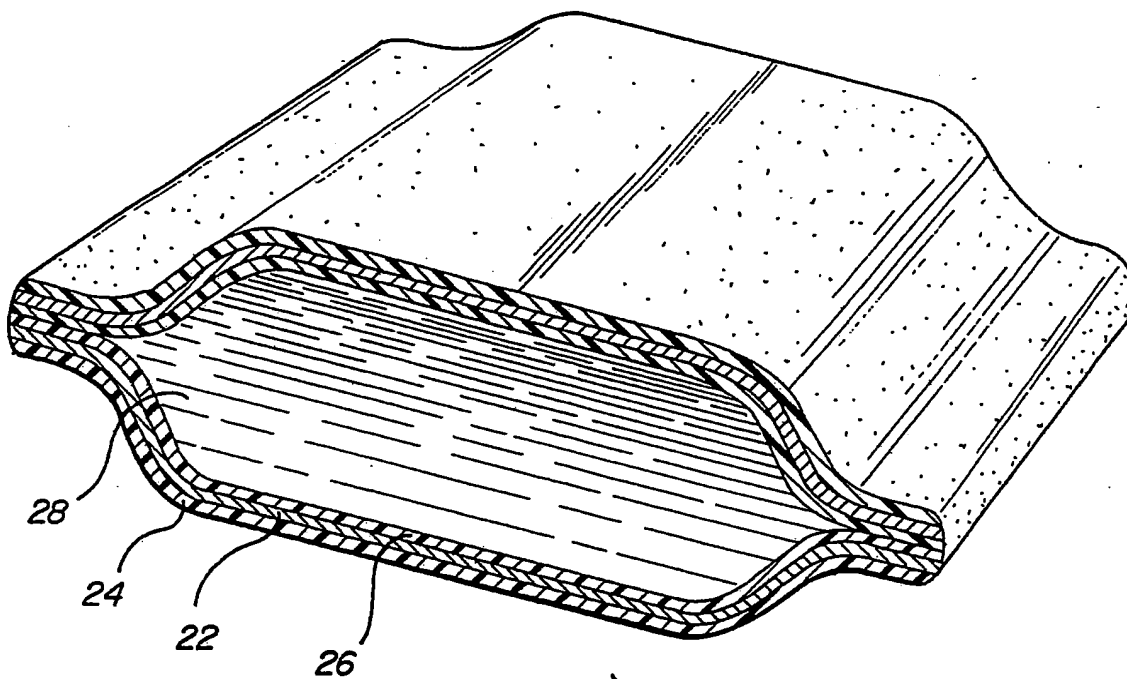
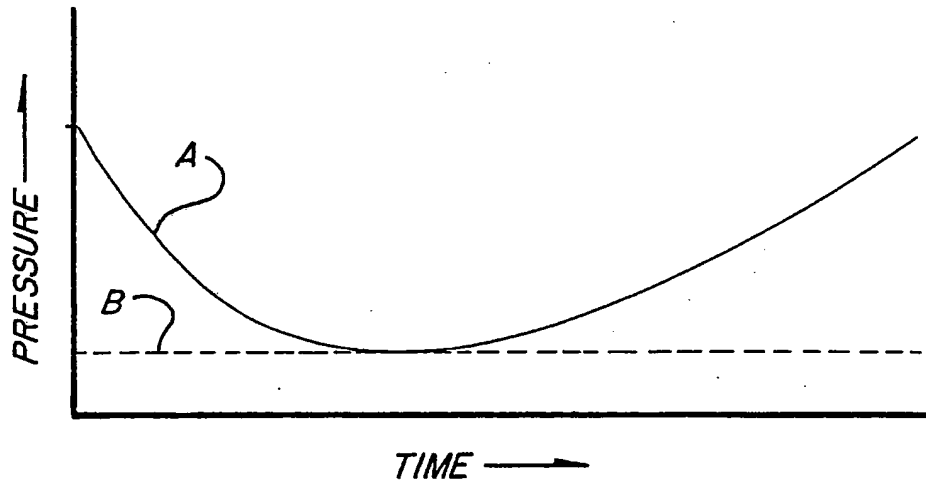
a liquid vehicle buffered to maintain a pH in the range of 6.8 - 7.5, disposed so as to completely fill said flexible container, with zero headspace;

oxygen dissolved in said vehicle in an amount
5 sufficient to create a partial pressure thereof in the range of 50-300 mmHg;

carbon dioxide dissolved in said vehicle in an amount sufficient to create a partial pressure thereof of 10-100 mmHg; and

10 helium dissolved in said vehicle, the total dissolved gas pressure of said reagent being no greater than 760 mmHg at 37°C.

1/1

FIG-1FIG-2

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/03603

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : G01N 33/96, 33/50, 31/00

US CL : 436/8, 9, 11, 16, 18, 19, 68; 252/408.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/8, 9, 11, 16, 18, 19, 68; 252/408.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, search term: helium

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US, A, 3,681,255 (Wilfore) 01 August 1972, see entire document.	1-10,12 <hr/> 11
X — Y	US, A, 4,960,708 (Zowtiak et al) 02 October 1990, see entire document.	1-10,12 <hr/> 11
Y	US, A, 4,786,394 (Enzer et al) 22 November 1988, see entire document.	1-12
A	US, A, 3,973,913 (Louderback) 10 August 1976, see entire document.	1-12
A	US, A, 4,116,336 (Sorensen et al) 26 September 1978, see entire document.	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,163,734 (Sorensen et al) 07 August 1979, see entire document.	1-12
A	US, A, 4,289,648 (Hoskins et al) 15 September 1981, see entire document.	1-12
A	US, A, 4,753,888 (Chiang) 28 June 1988, see entire document.	1-12
A	US, A, 4,871,439 (Enzer et al) 03 October 1989, see entire document.	1-12
A	US, A, 4,945,062 (Chiang) 31 July 1990, see entire document.	1-12
A	US, A, 5,023,186 (Herring) 11 June 1991, see entire document.	1-12